



## CASE STUDY

# Estimation of serum inflammatory cytokines( IL-4, IL 10 and IL 17) and total IgE concentrations in patients with bronchial asthma by ELISA technique.

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### Abstract

**This study aimed to determine the levels of immunomodulators by ELISA techniques . This study grouped asthmatic patients into three group of allergy modes according to concentration of total IgE measured by (ELISA ) in which it was found that mode of allergy (very probable) IgE>100 IU. The immunological parameters included: measurement of IL 4 ,10 and 17 . The study showed a high significant elevation of various interleukin 4,10 and 17 . IL-4 record elevated in age group 3 in male (11.40) and group 5 male (18), IL-10 record elevated in age group 4 female (12.30).IL-17 record record elevated in age group 2 female and 3 male(12.90).**

Keywords: ELISA, human bronchial asthma.interleukines

## 1 | INTRODUCTION

Asthma is a complex respiratory disease in which genetic predisposition, environmental and immunological influences interfere with each other (Edwards ,et al., 2012). It is considered one of the most prevalent chronic diseases, affecting approximately 300 million individuals (Masoli, et al., 2004) and causing an estimated 250,000 deaths each year (Bateman, et al., 2008). In addition, it is projected that by 2025, the global asthma burden will rise by 100 million people due to a growing Westernized lifestyle and urbanization in developing countries (Masoli, et al., 2004). The 'hygiene theory' was originally attributed to an increase in the prevalence of allergic diseases, including asthma, indicating that decreased exposure to microbes during the first years of life plays a role in the development of allergic diseases (Strachan, 1989, 2000). While

this theory is generally accepted, studies have shown that the increased incidence of asthma, rhinitis, or Neurodermitis does not completely account for decreased microbial exposure (Mallol, 2008; Brooks et al.,2013; Kramer et al., 2013). Asthma is a widespread illness globally and affects individuals of all ages, This condition usually occurs in infancy and is characterized by variable symptoms of wheeze, dyspnea, and chest tightness caused by air flow ob-

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ESTIMATION OF SERUM INFLAMMATORY CYTOKINES( IL-4, IL 10 AND IL 17) AND TOTAL IGE CONCENTRATIONS IN PATIENTS WITH BRONCHIAL ASTHMA BY ELISA TECHNIQUE

struction (fully reversible) (GINA, 2015; Bisgaard & Bonnelykke,2010).

**2 | MATERIALS AND METHODS**

**Samples**

A total of (312) patients (149 males and 163 females) of various age groups were included in this Case –control study. The patient was examined, and diagnosed as asthma under supervision of the Physician.the study was carried out during a period from July 2018 to January 2020.

**The grouping of patient**

Male& Female patients were divided into five groups according to (Falk, 1993; Herd,et al.,1996 ; Nishioka,1996 ; charman&Williams,2002)

**Group 1: 1- 11 years**

**Group 2: 12 – 2o years**

**Group 3: 21- 3o years**

**Group 4: 31 – 4o years**

**Group 5: above 4o years**

**Control group**

A total of (204) healthy individual (81 males and 123 females) with out any features of asthma or any allergic to be compared with asthmatic patient in genetic and immunological studies.

**TABLE 1:** Reagent of human IgE Elisa Kit (ICL)

| Reagent                         | Quantity and characters   |
|---------------------------------|---|
| Microtiter strip                | 12 MIC 8-well snap-off strips,coated with monoclonal Anti-IgE                             |
| Washing sol. Conc.              | 2*6o ml WS conc.for ca.12ooml PBS Puffer Tween 2o PH 6.5-7.o                              |
| Enzyme –antibody conjugate 1oox | one vial containing 22 ml of affinity purified anti-human IgE (goat)peroxidase -conjogate |
| Chromogen-substrate sol.        | one vial containing 13 ml of Substrate Reagent 3,3',5,5'- Tetramethylbenzidine(TMB)       |
| Stopping sol.                   | one vial containing 13 ml of o.5 mol/l sulfuric acid                                      |
| Adhesive strip                  | 2 Adhesive strip  |
| Human IgE calibrator            | Six vials containing human IgE Calibrator   |

**TABLE 2:** IL-4 Elisa Kit with pre-coated plates(Blo LEGEND MAX Human.USA)

| Content Description                                  | Quantity(1 plate) |
|--|-------------------|
| Anti human IL 4 pre-coated 96 –well Strip Microplate | 1 Plate           |
| Human IL-4 Detection Antibody                        | 1 bottle          |
| Human IL -4 Standard                                 | 1 Vial            |
| Matrix C (for serum and plasma sample only)          | 1 bottle          |
| Avidin-HRP B   | 1 bottle          |
| Assay buffer   | 1 bottle          |
| Wash buffer (2o x)                                   | 1 bottle          |
| Substrate solution F                                 | 1 bottle          |
| Stop solution  | 1 bottle          |
| Plate sealers(4 sheets)                              | 1 pack            |

**TABLE 3:** IL-1o Elisa Kit with pre-coated plates (BioLEGEND MAX Human.USA)

| Content Description                                   | Quantity (1 plate) |
|---|--------------------|
| Anti human IL 1o pre-coated 96 –well Strip Microplate | 1 Plate            |
| Human IL-1o Detection Antibody                        | 1 bottle           |
| Human IL -1o standard                                 | 1 Vial             |
| Avidin-HRP A  | 1 bottle           |
| Assay buffer  | 1 bottle           |
| Wash buffer (2o x)                                    | 1 bottle           |
| Substrate solution F                                  | 1 bottle           |
| Stop solution   | 1 bottle           |
| Plate sealers(4 sheets)                               | 1 pack             |

**TABLE 4:** IL-17 AElisa Kit with pre-coated plates(Blo LEGEND MAX Human. USA)

| Content Description                                     | Quantity(1 plate) |
|---|-------------------|
| Anti human IL 17 A pre-coated 96 –well Strip Microplate | 1 Plate           |
| Human IL-17 A Detection Antibody                        | 1 bottle          |
| Human IL -17 A standard                                 | 1 Vial            |
| Avidin-HRP D  | 1 bottle          |
| Assay buffer  | 1 bottle          |
| Wash buffer (2o x)                                      | 1 bottle          |
| Substrate solution F                                    | 1 bottle          |
| Stop solution   | 1 bottle          |
| Plate sealers (4 sheets)                                | 1 pack            |

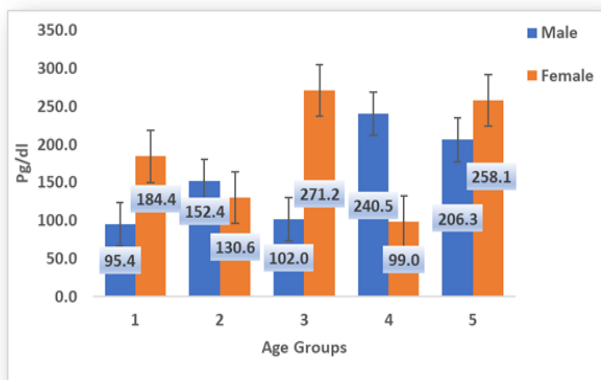
### 3 | RESULTS

#### Total IgE

Total concentration of IgE in Asthmatic patient sera of various age group illustrate in figure (1)

Age groups were recorded the following concentration for male and female respectively (95.4,184.4),(152.4,130.6), (102.0,271.2),(240.5,99.0)

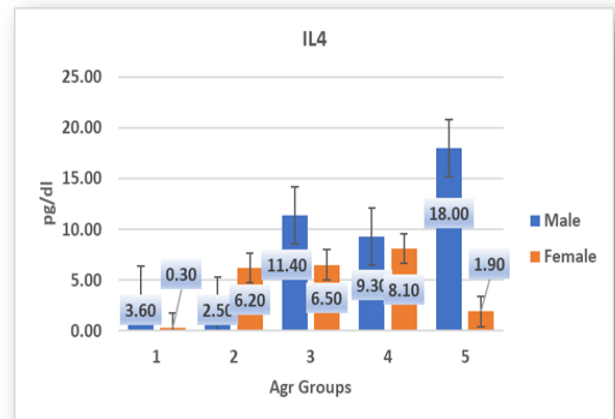
and (206.3,258.1)IU/ml for age groups 1,2,3,4 and 5 respectively there are a significant differences ( $p < 0.05$ ) between concentration of various age groups and between male and female the concentration of Total IgE in healthy person was 20-100 IU/ml (Allergy questionable) above 100 IU/ml (Allergy very propable)



**FIGURE 1:** Total IgE concentration in various age groups of Asthmatic patient  $P < 0.05$

#### IL-4

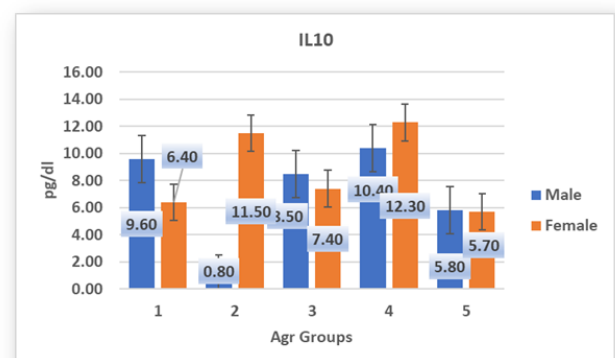
The concentration of IL-4 in Asthmatic patient sera in various age groups is illustrated in Fig.(3-4)for both male and female as follow.(3.6,0.3),(2.5,6.2),(11.4,6.5),(9.3,8.1) and (18,1.9) pg/dl for age groups 1,2,3,4 and 5 respectively. The standard concentration of IL-4 in healthy person was(4.5-9.6)pg/dl there on astatically differences between a concentration of various age groups ( $P < 0.05$ ).



**FIGURE 2:** Concentration of IL-4 in various age groups of Asthmatic patients  $P < 0.05$

#### IL-10

The concentration of IL-10 in asthmatic patient for each male and female in various groups is illustrated in figure (3) as follows: (9.6,6.4),(0.8,11.5),(1.5,7.4),(10.4,12.3) and (5.8,5.7) pg/dl for various age groups 1,2,3,4 and 5 respectively the standard concentration for IL-10 in healthy person were between (4.8-9.8) pg/dl there are significant differences between all studies concentration of IL-10 in age groups for both male and female ( $P < 0.05$ )



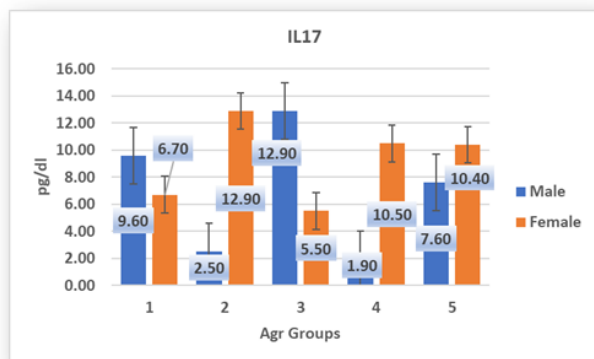
**FIGURE 3:** Concentration of IL-10 in various age group of Asthmatic patients  $P < 0.05$

#### IL-17

The concentration of IL-17 was studied in Asthmatic patient sera for male and female of various age groups and illustrate in Fig.(3-6) as follows:

## ESTIMATION OF SERUM INFLAMMATORY CYTOKINES( IL-4, IL 10 AND IL 17) AND TOTAL IGE CONCENTRATIONS IN PATIENTS WITH BRONCHIAL ASTHMA BY ELISA TECHNIQUE

(9.6,6.7),(2.5,12.9),(12.9,5.5),(1.9,10.5) and (7.6,10.4) pg/dl for age groups 1,2,3,4 and 5 respectively the standard value of IL-17 concentration was (6.26 – 7.2) pg/dl, There are statistical differences between concentration of IL-17 in both male and female of all studied age groups( $P < 0.05$ )



**FIGURE 4:** Concentration of IL-17 in various age groups of Asthmatic patient  $P < 0.05$

### 4 | DISCUSSION

Interleukin 4 stimulates activated B-cell and T-cell proliferation . Also B cells differentiation and the class switching to IgE . Regarding IL-4 ,record elevated in age group 3 female (13.2 pg) and male (11.7 pg), group 4 female (12.1pg)and group 5 female (11.3 pg) male (10 .2 pg) researcher believe IL-4 plays a crucial role in type 2 T-helper responses and isotype class switching of B cells to IgE synthesis, and it has thus been suggested that IL-4 may have an important role in asthma pathogenesis IL-4 expression in this situation may simply reflect a surrogate marker of “Th2-type” T-cell activation, Our result confirmed by (Russell,R,2020.;Renauld,2001;jian *et al.*,2000;Kianmehr,M *et al.*,2019) .While other study not confirmed (Mazloomi,E,*et al.*2018;Saadat,S.*et al.*2020;Kianmehr,M. *et al.*,2017).

IL-10 as an important risk gene for asthma (Nie W ,*et al.*, 2012; Hyun M.*et al.*,2013) Regarding IL-10,record elevated in age group 3 female (13.4 pg) and group 4 male (10.2) and female (10.5) Our result

confirmed by (Mahaki ,H.*et al.*,2020; Mokhtari-zezar,*et al.*, 2020; Hajdu,Z.*et al.*, 2018) other study not agreed(Mustafa,2020;Ahmadi,M.*et al.*,2017).

IL-17 immunity has been associated with asthma exacerbations.( Favata, *et al.*2017; Mutters, *et al.*2017).Regarding IL-17 Majority in age group 3 male (12.90 pg) female (14.2 pg), researcher believe allergic asthma may be influenced by the IL-17 levels .Our result confirmed by (Kianmehr,M.*et al.*,2019;Guerra,E.2016;Saunders,S.P,*et al.*,2019).other result not confirmed (Saadat,S.*et al.*,2020;Chehimi,M.*et al.*,2017;Kianmehr.M.*et al.*,2017).

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